University of Alberta

Effect of Exercise Training on Natural Killer Cell Cytotoxic Activity in Postmenopausal Breast Cancer Survivors: Results From the REHAB (Rehabilitation Exercise for Health After Breast Cancer) Randomized Controlled Trial

By

Adrian Stuart Fairey

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

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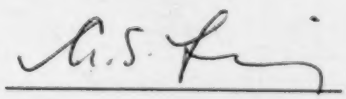

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Effect of Exercise Training on Natural Killer Cell Cytotoxic Activity in Postmenopausal Breast Cancer Survivors: Results From the REHAB (Rehabilitation Exercise for Health After Breast Cancer) Randomized Controlled Trial* submitted by *Adrian Stuart Fairey* in partial fulfillment of the requirements for the degree of *Master of Science*.

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Date Approved: 9 April 2002

DEDICATION

This thesis is dedicated to my parents, Dr. Daphne Fairey and Dr. Nigel Fairey, and to my significant other, Dr. Lise Warmington. Your support, encouragement, and patience were an integral part of the success of this thesis.

ABSTRACT

Purpose: To determine the effect of exercise training on natural killer cell cytotoxic activity in postmenopausal breast cancer survivors.

Methods: Fifty-three postmenopausal breast cancer survivors were randomly assigned to either an exercise (n=25) or control (n=28) group. The exercise group trained on cycle ergometers three times per week for 15 weeks. The control group did not train. Natural killer cell cytotoxic activity was assessed at baseline and postintervention using a ^{51}Cr release assay.

Results: Change score analyses showed significant and borderline significant differences favouring the exercise group in percent specific lysis of a natural killer cell sensitive cell line at the 3.125:1 (p=.011), 25:1 (p=.088), and 50:1 (p=.090) effector-to-target ratios. Between group analyses showed no group differences at baseline whereas at postintervention the exercise group had significantly higher percent specific lysis at the 3.125:1 (p<.001), 6.25:1 (p=.007), 12.5:1 (p=.011), and 25:1 (p=.045) effector-to-target ratios, and higher lytic activity per cell (p=.035). Within group analyses showed that the exercise group had a significant increase in percent specific lysis at the 3.125:1 (p=.003), 6.25:1 (p=.022), and 12.5:1 (p=.037) effector-to-target ratios, and a borderline significant increase in lytic activity per cell (p=.054) whereas there were no changes in the control group.

Conclusion: Exercise training had a beneficial effect on natural killer cell cytotoxic activity in postmenopausal breast cancer survivors who had completed therapy.

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TABLE OF CONTENTS

Contents	Page #
Chapter 1: Background	
1.1 The Rehabilitation Exercise for Health After Breast Cancer Trial.	2
1.2 References	4
Chapter 2: Literature Review	
2.1 Literature Search Topics	6
2.2 Literature Search Strategies and Data Extraction	6
2.3 Breast Cancer Epidemiology	6
2.4 Breast Cancer Staging Classification System	7
2.5 Breast Cancer Treatments and Their Side Effects	7
2.6 The Immune System	7
2.7 Immune System Response to Tumors	11
2.8 Breast Cancer Treatment and the Immune System	13
2.9 Immunity and Clinical Outcome in Breast Cancer Survivors	13
2.10 Exercise Training and the Immune System in Cancer Survivors	14
2.10.1 Purposes and Hypotheses	14
2.10.2 Samples and Designs	15
2.10.3 Physical Exercise Training Interventions	16
2.10.4 Assessments	16
2.10.5 Study Results	17
2.10.6 Limitations and Future Research Directions	18
2.10.7 Sample Limitations and Future Research Directions	18

2.10.8 Design Limitations and Future Research Directions..... 21

2.10.9 Physical Exercise Training Intervention Limitations and Future Research Directions..... 21

2.10.10 Assessment Limitations and Future Research Directions... 22

2.10.11 Other Recommendations for Future Research..... 25

2.11 References..... 27

Chapter 3: Effect of Exercise Training on Natural Killer Cell Cytotoxic Activity in Postmenopausal Breast Cancer Survivors: Results From the REHAB (Rehabilitation Exercise for Health After Breast Cancer) Randomized Controlled Trial

3.1 Background..... 42

3.2 Methods..... 44

3.3 Results..... 53

3.4 Discussion..... 57

3.5 References..... 62

Chapter 4: General Discussion

4.1 Key Finding..... 76

4.2 Strengths and Limitations of the Trial Design..... 76

4.3 Directions for Future Research..... 77

4.4 Long-Term Exercise Adherence..... 78

4.5 Summary..... 78

4.6 References..... 79

Appendix

5.1 Reference Ranges for White Blood Cell Counts and Differentials..... 81

LIST OF TABLES

Table	Page #
Chapter 2	
Table 2.1: The American Joint Committee on Cancer Classification System	34
Table 2.2: Breast Cancer Treatments and Their Side Effects	35
Table 2.3: Human Tumor Antigens Associated with the Antitumor Response	36
Table 2.4:Cellular Immune System Components Involved in Cancer Defence	37
Table 2.5: Soluble Factors of the Immune System Involved in Cancer Defence	38
Table 2.6: Summary of Studies Examining the Effects of Physical Exercise Training on Immune System Function in Cancer Survivors	39
Chapter 3	
Table 3.1: Baseline Characteristics	67
Table 3.2: Blood Collection Protocol Information	68
Table 3.3: Change Scores Analyses for the Effects of Exercise Training on Natural Killer Cell Activity	69
Table 3.4: Between Group Analyses for the Effects of Exercise Training on Natural Killer Cell Activity	70
Table 3.5: Within Group Analyses for the Effects of Exercise Training on Natural Killer Cell Activity	71
Table 3.6: Change Scores Analyses for the Effects of Exercise Training on White Blood Cell Counts and Differential	72
Table 3.7: Between Group Analyses for the Effects of Exercise Training on White Blood Cell Counts and Differential	73
Table 3.8: Within Group Analyses for the Effects of Exercise Training on White Blood Cell Counts and Differential	74

LIST OF FIGURES

Figure	Page #
Chapter 2	
Figure 2.1: The Innate Immune System.....	9
Figure 2.2: The Acquired Immune System.....	10

LIST OF ABBREVIATIONS

ADHE	Anti D-Loaded Human Erythrocytes
BSA	Bovine Serum Albumin
CD	Cluster of Differentiation
CI-MPR	Mannose-6-Phosphate/Insulin-Like Growth Factor Receptor
CTL	Cytotoxic T Lymphocyte
EDTA	Ethylenediamine Tetraacetic Acid
E:T	Effector-to-Target Ratio
g	G Force
GrB	Granzyme B
HR	Heart Rate
HRmax	Maximum Heart Rate
IGF	Insulin-Like Growth Factor
IFN	Interferon
IL	Interleukin
LU	Lytic Units
NKCA	Natural Killer Cell Cytotoxic Activity
PBS	Phosphate Buffered Saline
PI	Phagocytosis Index
RSDE	Receptor Destroying Enyzme-Treated Sheep Erythrocytes
REHAB	Rehabilitation Exercise for Health After Breast Cancer
TATA	Tumor-Associated Transplantation Antigen
TNF	Tumor Necrosis Factor
TSTA	Tumor-Specific Transplantation Antigen
y	Year
^{51}Cr	Sodium Chromate Labelled Chromium
↑	Increase
↓	Decrease
↔	No Change
*	Not Statistically Significant

CHAPTER 1

Background

1.1 The Rehabilitation Exercise for Health After Breast Cancer Trial

The Rehabilitation Exercise for Health After Breast Cancer (REHAB) trial was a randomized controlled trial designed to determine the effects of supervised exercise training on cardiopulmonary, quality of life, and biologic outcomes in postmenopausal breast cancer survivors who had completed therapy. Cardiopulmonary outcomes included oxygen consumption and power output at peak exercise, oxygen consumption and power output at the ventilatory equivalent for oxygen, and oxygen consumption and power output at the ventilatory equivalent for carbon dioxide. Quality of Life outcomes included multidimensional quality of life, happiness, fatigue, and self-esteem. Immunologic outcomes included natural killer cell cytotoxic activity, immune cell phenotype levels, white blood cell counts and differentials, lymphocyte proliferation, neutrophil function, and cytokine production. Metabolic hormone outcomes included glucose, insulin, insulin-like growth factor-1, and insulin-like growth factor binding protein-3. Sex steroid hormone outcomes included estradiol, estrone, testosterone, androstenedione, dehydroepianodrosterone, dehydroepianodrosterone sulfate, and sex hormone binding globulin. Lipid outcomes included total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, and triglycerides.

Cardiopulmonary and quality of life outcomes from the REHAB trial have been reported previously.[1] We found significant differences favoring the exercise group in oxygen consumption and power output at peak exercise, oxygen consumption and power output at the ventilatory equivalent for oxygen, and

oxygen consumption and power output at the ventilatory equivalent for carbon dioxide. Concomitant significant differences were found for multidimensional quality of life, happiness, fatigue, and self-esteem.

The current thesis reports the effect of exercise training on selected immunologic outcomes from the REHAB trial. The primary outcome was natural killer cell cytotoxic activity. Secondary outcomes were the percentage of natural killer cells and white blood cell counts and differentials. Effects on the metabolic hormone, sex steroid hormone, and lipid outcomes will be reported separately.

1.2 References

1. Courneya KS, Mackey JR, Bell GJ, Jones LW, Field CJ, Fairey AS. Randomized trial of exercise training in postmenopausal breast cancer survivors: cardiopulmonary and quality of life outcomes. Submitted to the Journal of Clinical Oncology

CHAPTER 2

Literature Review

2.1 Literature Search Topics

Several topics were reviewed for the current thesis. These included: 1) breast cancer epidemiology; 2) breast cancer staging; 3) breast cancer treatments and their side effects; 4) the immune system; 5) the immune system response to tumors; 6) breast cancer treatment and the immune system; 7) the immune system and clinical outcome; and 8) exercise training and the immune system in cancer survivors.

2.2 Literature Search Strategies and Data Extraction

Literature searches of computer databases were performed for the period up to and including December 2001 (MEDLINE 1966-2001, EMBASE 1980-2001, CANCERLIT 1988-2001, Sport Discuss 1949-2001). The search strategy included common text words and Medical Subject Headings related to the literature search topics. MEDLINE and EMBASE searches were limited to human subjects. Medical journals (e.g., Journal of the American Medical Association, Journal of Clinical Oncology, Journal of the National Cancer Institute, The New England Journal of Medicine) and manual hand-searches of the reference lists from relevant articles were also searched for additional material. Non-English information was not reviewed. The author independently performed the literature searches and selected and extracted data.

2.3 Breast Cancer Epidemiology

An estimated 19,500 Canadian women will be diagnosed with breast cancer and 9,500 will die of breast cancer in 2001.[1] Canadian women have about an 11 percent lifetime probability of developing breast cancer and a 4 percent

lifetime probability of dying from breast cancer.[1] Survival after a diagnosis of breast cancer is dependent on whether the cancer is localized or has spread. The eight-year relative survival rate (adjusted for normal life expectancy) for women diagnosed with Stage I, II, III, and IV breast cancer is 90, 70, 40, and 10 percent, respectively.[2]

2.4 Breast Cancer Staging Classification System

Breast cancer is staged using the American Joint Committee on Cancer (AJCC) classification system.[3] It is based on tumor size, status of regional lymph nodes, and presence of distant metastasis. This staging system is presented in Table 2.1.

2.5 Breast Cancer Treatments and Their Side Effects

Early stage breast cancer is treated with surgery to remove the breast tumor. Many women are then recommended adjuvant therapy to reduce the risk of recurrent disease. Adjuvant therapies include radiotherapy, chemotherapy, and hormone therapy.[4] Unfortunately, these therapies are associated with several side effects.[5] A brief summary of current breast cancer treatments and their side effects is presented in Table 2.2.

2.6 The Immune System

The immune system protects against destructive forces either from outside the body (e.g., bacteria, viruses, and parasites) or from within (e.g., malignant and autoreactive cells). It comprises two functional divisions that work together in a coordinated manner. The innate immune system consists of cellular components, soluble factors, physical barriers, and the reticuloendothelial

system.[6] It provides a first line of defense against foreign pathogens while an acquired immune response is activated.[7] The acquired immune system produces a specific reaction and immunologic memory to each pathogen and comprises cellular components and soluble factors.[6] The innate and acquired immune systems are illustrated in Figures 2.1 and 2.2, respectively.

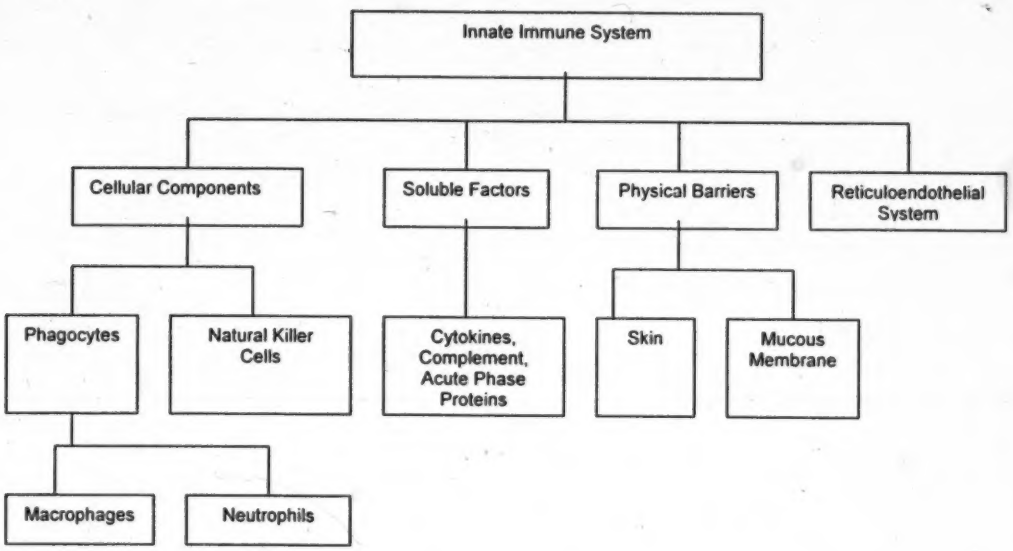

Figure 2.1. The Innate Immune System. The innate immune system is composed of cellular components, soluble factors, physical defenses, and the reticuloendothelial system that do not depend on previous exposure to a particular antigen. The cellular components of the innate immune system include phagocytes (macrophages, neutrophils) and natural killer cells. The soluble factors include cytokines, complement, and acute phase proteins. Physical barriers include the skin and mucous membranes. The reticuloendothelial system is comprised of phagocytic cells that have a primary role in alleviating blood-borne infections. Adapted from: Glodsby RA, Kindt TJ, Osborne BA. Kuby Immuology (4th ed.). New York: W.H. Freeman, 2000.

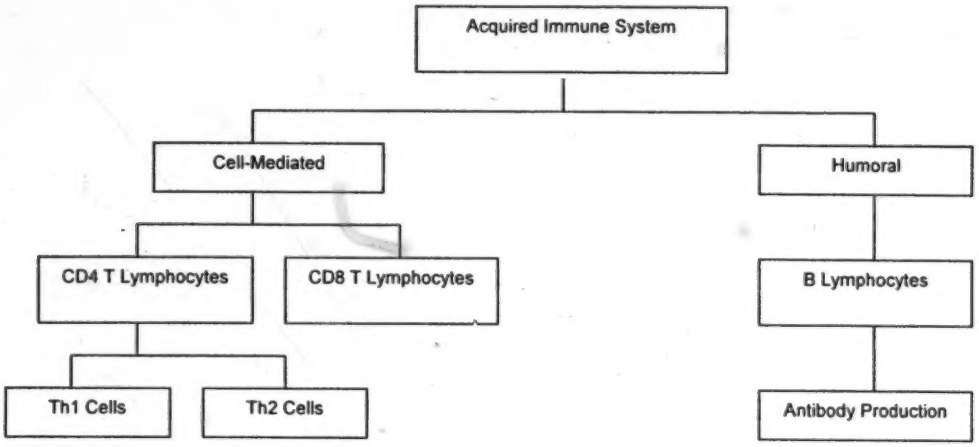

Figure 2.2. The Acquired Immune System. The acquired immune system is comprised of both a cell-mediated and humoral branch. The cell-mediated component consists of two types of T lymphocytes: CD4+ (helper/inducer) and CD8+ (cytotoxic/suppressor). CD4+ cells are important in both the cell-mediated and humoral immune response. The CD4+ subset is further divided into Th1 and Th2 immune cells based on the cytokines they produce and secrete. Th1 cells produce interleukin-2 and interferon-γ, whereas Th2 cells produce interleukin-4, 5, 6, 10, and 13. Cytotoxic T cells (CD8+) directly kill target cells by recognizing antigen in association with the MHC class I that is present on every cell in the human body. B cells are the major effector cells of the humoral system. They are activated to become antibody-secreting plasma cells. Adapted from: Glodsby RA, Kindt TJ, Osborne BA. Kuby Immuology (4th ed.). New York: W.H. Freeman, 2000

2.7 Immune System Response to Tumors

The theory of immune surveillance asserts that many potential cancers might be eradicated prior to their clinical detection if they can be recognized and destroyed by acquired and/or innate immune system components.[8,9] According to this theory, tumors arise only if cancer cells are able to escape or overcome immune surveillance. Transformed cells are able to avoid destruction by the immune system either by reducing their expression of tumor antigens or by an impaired or suppressed immune response to these cells.[6] Support for the theory can be found in research that has shown that the immune system may have a particularly integral role in malignancies of viral origin.[10] Researchers have demonstrated an increased incidence of specific cancers in immunosuppressed patients with AIDS. These cancers include non-Hodgkin's lymphoma, Kaposi's sarcoma, anal cancer, and cervical cancer.[11] Other findings, however, seem to challenge the tenants of the immune surveillance theory. For example, although individuals receiving immunosuppressive drugs show an increased incidence of immune system cancers, they do not demonstrate an increased incidence of other common cancers such as lung, breast, and colon.[6] Although the immune surveillance theory remains controversial, it is clear that both acquired and innate immune system components are able to produce an anticancer response to tumor cells.[6]

The acquired immune system must recognize tumor antigens in order to mount an anticancer T cell response. Tumor antigens located on tumor cells are either tumor-specific transplantation antigens (TSTA) and tumor-associated

transplantation antigens (TATA).[6] TSTA are unique to tumor cells and absent on normal cells. In contrast, TATA are not unique to tumor cells in that they are expressed on normal cells during fetal development but are usually not expressed in adults.[6] Tumor antigens recognized by human T cells can be classified as one of four types: 1) antigens encoded by genes explicitly expressed by tumors; 2) antigens encoded by deviate forms of normal genes that have been changed by mutation; 3) antigens typically expressed at certain stages of differentiation or only by certain differentiation lineages; and 4) antigens that are expressed in excess by certain tumors.[6] Although some human cancers have been shown to be nonimmunogenic, research is accumulating to suggest that a specific immune response may still be an important defense against these tumor cells.[6,9,12,13] Human tumor antigens that have been associated with the anticancer immune system response are presented in Table 2.3.

The innate immune system is also able to mount an anticancer response against tumor cells. Unlike the acquired immune system response, however, innate components can eliminate neoplastic cells without prior exposure to a particular antigen.[6] Evidence that innate immune system reactions to tumor cells are important in host defence can be found in studies that have examined their immunologic role in the maintenance of cancer quiescence during remission.[15] Animal research has also demonstrated that enhanced tumor clearance is associated with high levels of activity by innate immune components.[16,17] Cellular innate immune system components involved in anticancer defense include natural killer cells, macrophages, and neutrophils. Soluble factors of the innate

immune system implicated in cancer defence include C-reactive protein, interleukin 1, tumor necrosis factor, interferon, and oncostatin M.[6] A brief review of the cellular components and soluble factors of the immune system that are involved in the antitumor response is provided in Tables 2.4 and 2.5.

2.8 Breast Cancer Treatment and the Immune System

Breast cancer treatments can have a dramatic effect on the immune system. In general, although the results are not entirely consistent, anticancer therapies tend to be immunosuppressive. For example, immunologic variables that are reduced after chemotherapy include total lymphocyte counts, T cell counts (CD4+, CD8+), B cell counts (CD19+), natural killer cell counts (CD3–CD16+CD56+), activated T cell counts, and natural killer cell cytotoxic activity.[18,19] Immunologic indices that are impaired after radiotherapy include natural killer cell cytotoxic activity[20] and those that are compromised after surgical intervention include natural killer cell cytotoxic activity, monocyte phagocytosis, antigen presentation and superoxide release, B cell immunoglobulin production, T cell responses to mitogen stimulation, and interleukin-2 production.[21]

2.9 Immunity and Clinical Outcome in Breast Cancer Survivors

Little is known about the relationship between changes in immune function and important clinical outcomes in breast cancer survivors. Preliminary studies, however, have emerged. For example, Head et al. demonstrated that the magnitude of the decrease in the number of neutrophils and lymphocytes that occurred during chemotherapy was associated with disease relapse.[22] In addition, de Gast et al. showed that five out of seven patients who had a rapid

recovery of CD4+ and CD8+ T cells within one month after high-dose chemotherapy were relapse-free after 12 to 27 months of follow-up.[23] Finally, Demaria et al. found that the development of tumor infiltrating lymphocytes correlated with clinical response to neoadjuvant pacitaxel therapy.[24] Although further research is needed on this topic, there is preliminary evidence to suggest that antitumor immunity may be important to clinical response in breast cancer survivors.

2.10 Exercise Training and the Immune System in Cancer Survivors

We recently published a systematic review of the literature that has examined the effect of exercise training on immune function in cancer survivors.[25] Studies were included in our review if they met the following two criteria: 1) examined an aerobic and/or resistance exercise training intervention designed to improve cardiopulmonary fitness and/or muscular strength in human cancer survivors and 2) identified the immunologic outcome that was studied and the protocol used to assess it.

Six research articles published between 1994 and 2000 were found. Table 2.6 summarizes information from the six studies including an extensive overview of the samples, designs, physical exercise training interventions, assessments, and results separated by timing of the intervention. An overview of these parameters is presented below.

2.10.1 Purposes and Hypotheses

All six studies examined the effects of physical exercise training on the immune system in cancer survivors during or after cancer treatment.[26-31] The

hypotheses tested in the six studies were similar. Specifically, the investigators hypothesized that moderate intensity physical exercise training would improve immune system function in cancer survivors during or after cancer treatment.[26-31]

2.10.2 Samples and Designs

Participants in the six studies varied. Two out of six studies examined cancer survivors during treatment,[26,31] whereas four studies examined cancer survivors posttreatment.[27-30] Two studies examined the same sample of breast cancer survivors who had Stage I or II disease,[28,29] while another study examined a different sample of breast cancer survivors.[30] The other studies investigated autologous peripheral blood cell transplant survivors with solid tumors,[26] stomach cancer survivors,[27] and children with a history of acute lymphoblastic leukemia, Ewing's Sarcoma, or Non-Hodgkin's lymphoma.[31] Three out of six studies examined females,[28-30] one study investigated both males and females[26] and two studies did not report the gender of their subjects.[27,31] The sample sizes for the studies ranged from 6[31] to 70[26] with a mean of 28. One study performed a statistical power calculation to determine the number of subjects required to detect a clinically and statistically significant difference in the primary endpoint.[26]

Study designs in the six studies also varied. Three studies were randomized controlled trials with usual care controls,[26,27,30] two studies were a pretest-posttest design with no controls,[28,29] and one study was a pretest-posttest design with matched controls (i.e., non-exercising cancer survivors and exercising healthy persons).[31]

2.10.3 Physical Exercise Training Interventions

Several physical exercise interventions were utilized. The frequency of the exercise was five times/week for two studies[28,29] and three or four times/week for an additional two studies.[30,31] Other studies reported an exercise training frequency of seven times/week[26] and 10 times/week.[27] The intensity of the exercise was between 60% and 80% of each subjects' maximum heart rate in five out of six studies.[26,28-31] One study did not provide the exercise training intensity.[27] The time spent on exercise during each training session was between 30 and 40 minutes in five studies[26-29,31] and 60 minutes in one study.[30] The length of the exercise interventions were 29 weeks,[28,29] 12 weeks,[31] eight weeks,[30] or two weeks.[26,27] The primary exercise mode in three out of six studies was cycle ergometer.[26,28,29] One study combined walking and resistance training exercise,[30] while one study combined range of motion exercises, pelvic tilting exercises, isometric quadriceps-setting exercises, and cycle ergometer.[27] The other study provided the subjects with a choice of several aerobic activities.[31] The exercise was supervised for the entire duration in four studies,[26,27,30,31] whereas two studies provided supervision for the first five weeks of the intervention.[28,29]

2.10.4 Assessments

Several physical fitness assessments were used to document an effect of exercise training. Three out of six studies used a graded or progressive bicycle ergometer test until exhaustion,[28,29,31] whereas one study used a graded treadmill stress-test until exhaustion.[26] One study used a combination of a symptom-

limited treadmill exercise test, 16-minute walk test, and leg extension strength test.[30] The type of physical fitness assessment was not reported in one study.[27]

A variety of cellular immune system components were assessed. All six studies obtained immune cells from samples of peripheral blood.[26-31] One study assessed natural killer cell (CD56+) counts and activity,[28] while one study each measured natural killer cell activity[27] and neutrophils.[26] One study assessed all leukocytes including lymphocyte, monocyte and granulocyte populations,[29] whereas one study measured all leukocytes including the number of natural killer cells (CD56+), T cells (CD3+, CD4+, CD8+, CD25+, CD122+), and B cells (CD19+).[31] One study assessed all leukocytes including the number of T cells (CD3+), natural killer cells (CD3–CD16+CD56+), and granulocytes.[30]

Several immunologic protocols were used to assess immune function. Three studies employed a combination of direct immunofluorescence and flow cytometry.[28,30,31] One study used a combination of a blood smear and functional test for the phagocytotic ability of monocytes,[29] while one study used complete blood counts.[26] Four studies utilized the ^{51}Cr release assay[27,28,30,31] and one study used PHA- and PWM-induced lymphocyte proliferation techniques.[31]

2.10.5 Study Results

The studies reported favorable physical fitness outcomes. Participants assigned to the physical exercise training group had lower heart rates during a fixed submaximal load, increased 16-minute walk distances, and increased leg strength compared to the control group after the exercise intervention.[30] Trained subjects compared to untrained had a decreased loss of physical performance[26]

and increased aerobic power.[31] Three studies did not report changes in cardiopulmonary fitness and/or muscular strength.[27-29]

The studies also reported favorable immunologic outcomes. Four out of six studies reported statistically significant improvements in immune function as a result of exercise. The immunologic benefits that were demonstrated included improvements in natural killer cell activity,[27,28] monocyte function,[29] proportion of circulating granulocytes,[29] and duration of neutropenia.[26] In contrast, two studies found no statistically significant improvements in immune function as a result of exercise. Nieman et al. found no statistically significant change in natural killer cell activity or the proportion of T and natural killer cells,[30] while Shore and Shephard reported non-significant decreases in T cell populations as a result of exercise.[31]

2.10.6 Limitations and Future Research Directions

Several limitations of the studies were identified. Moreover, there are many unexplored areas that warrant further investigation. Limitations of past research and directions for future studies are presented below.

2.10.7 Sample Limitations and Future Research Directions

Four important sample limitations were identified. First, all six studies used convenience samples.[26-31] This method of sampling is problematic in that there is no defined population from which the sample is drawn. As a result, the generalizability of the findings is limited because it is difficult to estimate the amount and nature of the selection bias that may exist in the sample. Second, five out of six studies collected data using small numbers of subjects.[27-31] For

example, in those five studies, an average of 20 subjects were assessed, of which 10 were included within the experimental condition. Small numbers of subjects reduces the power of a study and precludes the use of multivariate statistical techniques.[32] In addition, only one study performed a statistical power calculation to determine the number of subjects required to detect a clinically and statistically significant difference between groups in the primary endpoint.[26] Consequently, future exercise immunology research should attempt to recruit larger, random samples from a defined population of cancer survivors. Researchers should also give sufficient attention to statistical power in the planning stages of research to ensure that they will be able to detect a significant difference between groups in the primary endpoints, if one does indeed exist.

Third, subjects within all six studies were heterogenous with respect to one or more characteristic (s) including age, gender, and/or cancer site. Importantly, research has shown that several components of the immune system are significantly influenced by such variables.[33,34] For instance, increasing age has been associated with decreases in the total number of peripheral blood lymphocytes and T cells (CD4+, CD8+), T cell (CD45RA+) function, T cell responses to mitogenic stimulation, IL-2 production by T cells, and natural killer cell activity, to name a few.[35] In addition, four studies did not report information about the stage of cancer,[26,27,30,31] while five studies did not provide data regarding subject treatment protocols.[27-31] Therefore, future exercise immunology research should attempt to recruit homogenous samples of cancer survivors to reduce misinterpretations of any exercise-induced immune system response.

Researchers must also describe, in sufficient detail, information regarding the phase of treatment as has been done to a limited extent in one study.[26] In particular, researchers should attempt to identify cancers using the TNM classification system, which assesses tumors in three ways: extent of the primary tumor (T), absence or presence of regional lymph node involvement (N), and absence or presence of distant metastases (M).[3] In addition, investigators should delineate specific details regarding the treatment protocols (e.g., mastectomy and axillary node dissection, radiotherapy, chemotherapy, and hormonal therapy) and the timing of these interventions in relation to exercise (i.e., exercise during cancer treatment versus posttreatment). Due to the profound and prolonged immune effects of allogenic red blood cell transfusions[36] commonly required in the perioperative period or during chemotherapy administration, transfusion data must also be obtained. Such information will allow exercise immunologists to accurately compare exercise-induced immune system alterations and, ultimately, help to reduce misinterpretation of the immune response in cancer survivors.

Fourth, three out of six studies have examined breast cancer survivors,[28-30] while one study each assessed mixed cancer survivors recovering from autologous peripheral blood stem transplant,[26] children with mixed cancers[31], and stomach cancer survivors.[27] The unique demographics, pathology, surgical procedures, and treatment protocols make it unwise to generalize the results from one cancer site to another. Thus, future exercise immunology research should examine common cancers such as prostate, lung, colon, kidney, bladder,

uterine and, in particular, malignancies that have been shown to have a strong association with the immune system (e.g., melanoma).

2.10.8 Design limitations and Future Research Directions

One important design limitation was identified. Two out of six studies utilized a pretest-posttest design with no controls[28,29] and one study used a pretest-posttest design with matched controls.[31] These designs are problematic in that they leave findings potentially vulnerable to numerous threats to internal validity (e.g., circadian and seasonal changes in immune system function).[37] Therefore, future exercise immunology research should attempt to use randomized controlled trials with usual care controls or wait-list controls, which will help to reduce the influence of confounding variables.[32] Randomized controlled trials have also been proposed as the strongest design by which to examine biomarkers in exercise and cancer research.[38]

2.10.9 Physical Exercise Training Intervention Limitations and Future Research Directions

Two main limitations of the physical exercise interventions were identified. First, three out of six studies prescribed an intervention that lasted for a period of less than 12 weeks.[26,27,30] This intervention period is relatively short, particularly if the intent is to examine the effect of physical exercise training on immune system function, and subsequent risk of cancer recurrence/secondary malignancy and survival. Thus, future exercise immunology research should attempt to assess long-term exercise training interventions (i.e., 6 or 12 months). Second, three out of six studies employed partially unsupervised training interventions.[28,29,31] Self-

report measures of physical exercise may lead to inaccurate data regarding the frequency, intensity, and duration of exercise that was performed.[39] Therefore, future exercise immunology research should attempt to employ supervised exercise interventions with close monitoring of exercise parameters.

2.10.10 Assessment Limitations and Future Research Directions

Two limitations of the physical fitness assessments were identified. One study did not provide any information about the physical fitness assessment (s).[27] Clearly, it is imperative that exercise immunology studies in cancer survivors provide such information. Second, two studies performed a preintervention assessment but no postintervention assessment.[28,29] Accordingly, future exercise immunology research should attempt to assess cardiopulmonary fitness and/or muscular strength before and after the exercise intervention to document changes in physical fitness.

Seven main limitations of the immune system components and methods were identified. First, no study has related exercise-induced alterations in immune system function to important clinical outcomes for cancer survivors. Examples of germane clinical outcomes include toxicity of anticancer treatment, risk of recurrence and/or death, and the onset of late effects. Accordingly, future exercise immunology research in cancer survivors should attempt to correlate changes in immune system function to meaningful clinical outcomes.

Second, immune cells from all six studies were characterised using samples of peripheral blood. Peripheral blood samples may not be representative of the condition of the whole body since a large percentage of all leukocytes are

normally found outside of the circulating peripheral blood.[40] As a result, exercise immunologists should be cautious in the interpretation of such data if they are used to predict immune system component alterations in the lymphoid organs.[40] In addition, future exercise immunology research should attempt to establish whether exercise-induced alterations in immune system components from peripheral blood samples are similar to those obtained from the lymphoid tissues and/or other body fluids. Animal models may be helpful in this regard.[41,42]

Third, blood collection protocols that were used across the six studies were somewhat inconsistent. For example, five out of six studies did not provide dietary intake information on the days prior to blood collection.[26,28-31] Moreover, no study provided information pertaining to medications and/or sleeping patterns prior to blood collection. Because cellular and humoral immune system components are dramatically influenced by diet and nutritional status,[43,44] medication,[34] sleep patterns,[34] and smoking status,[34] subject behavior prior to blood collection is an obvious source of variability. Consequently, future exercise immunology research should attempt to establish standards for subject behavior in the periods several days before blood collection and during the time-course of the sampling procedure itself.[45] Such protocols may ask participants to consume the same diet on the three days prior to each blood collection session throughout the study.

Fourth, three out of six studies assessed immune system components before and after the intervention,[26,30,31] whereas three studies measured immune system function before, at some point during, and after the intervention.[27-29] As a

result, there is a paucity of information regarding alterations in immune system components during the exercise intervention. Future exercise immunology research should attempt to assess immune system function at several time points throughout the intervention (i.e., multiple time point analysis). Such a regimen may include assessments prior to program initiation, at program midpoint, at program endpoint, and post-program follow-up.

Fifth, four out of six studies did not report the time at which blood was drawn in relation to exercise.[27-30] One study each collected blood specimens 12 hours[26] and 36 hours[31] after the final exercise session. Given that the effect of exercise on the immune system both during and after an exercise bout is still unknown, future exercise immunology research should report the time at which blood specimens are collected. Studies should also attempt to collect blood specimens and thus measure immune system function at several time points during and following completion of exercise.

Sixth, several cancer-related immune system components have yet to be examined. Phenotypic analysis to determine the absolute numbers and/or percentages of leukocyte sub-populations and lymphocyte subsets were relatively limited and the influence of exercise on several immune cell types remains to be determined. In addition, only one study examined lymphocyte proliferation in response to antigenic stimulation[31] and no study assessed the oxidative capacity of neutrophils or B cell function. No study assessed acute phase proteins (i.e., C-reactive protein) and/or cytokines (IL-1, IL-6, IFN-α, IFN-γ, and TNF-α), all of which may be important in anticancer defense[6] and several of

which are influenced by exercise.[46] Therefore, future exercise immunology research should attempt to assess a wide variety of cancer-related immune system components. Phenotype analysis, functional assays, and cytokine analysis should be incorporated into future studies.

Seventh, the presentation of immune system results was somewhat inconsistent with current guidelines in exercise immunology.[45] For instance, natural killer cell activity assay results can be presented at single or multiple effector cell to target cell (E:T) ratio (s). Two out of six studies presented results using one E:T ratio,[27,28] while one study each presented results using two E:T ratios[30] and three E:T ratios.[31] Multiple ratios allow the researcher to present results as lytic units (LU) and single ratios can be influenced by the position of the titration curve.[47] Therefore, future exercise immunology research should attempt to present natural killer cell assay results at a minimum of two, but preferably, three or more E:T ratios.

2.10.11 Other Recommendations for Future Research

Research suggests that the effects of physical exercise on immune function may be mediated by a number of potential mechanisms. These include neuroendocrine hormones,[48-50] elevated body temperature,[51] energy sources,[52] and autocrine or paracrine molecules.[53] Recently, neuroendocrine components have received the most research attention in the exercise immunology literature. Foremost among these components are catecholamines, growth hormone, cortisol, β-endorphin, and sex steroids.[50-54] Given the diverse interactions between these biologic systems and their potential role in cancer defense,

exercise immunologists should be aware of and consider the impact of these complex interactions in future exercise immunology trials in cancer survivors. Knowledge of fundamental immunology, endocrinology, cell biology, and nutrition may help exercise immunologists to more fully appreciate the role of physical exercise in reducing the risk of cancer recurrence/secondary malignancy and increasing survival.

As one example, cytotoxic T lymphocytes (CTL) have been shown to mediate tumor cell apoptosis via granule-mediated exocytosis.[55] According to this mechanism, an interaction between granzyme B (GrB) and the mannose 6-phosphate/insulin-like growth factor receptor (CI-MPR) is essential for cell surface binding, uptake, and induction of tumor cell apoptosis.[56] Interestingly, insulin-like growth factors (IGF) have been shown to stimulate the release of CI-MPR from breast cancer cell lines and metastatic breast cancer cells.[57] An IGF-induced reduction in the number of CI-MPR on breast cancer cells may mean that there are fewer GrB – CI-MPR interactions, reducing the susceptibility of breast cancer cells to CTL-induced apoptosis. Importantly, physical exercise has been shown to reduce IGF-1. For example, Schmitz et al. reported that a 15-week resistance exercise program resulted in decreased IGF-1 in female subjects despite increased lean mass.[58] By inference, exercise-induced decreases in IGF-1 may decrease the release of CI-MPR from breast cancer cells, increase the number of GrB – CI-MPR interactions between CTL and breast cancer cells, and promote CTL-induced cancer cell apoptosis. Mechanistic studies of exercise-induced cancer control are needed.

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TABLE 2.1. The American Joint Committee on Cancer Breast Cancer Classification System[3]

Classification	Description
Primary Tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor ≤ 2 cm in greatest dimension
Tmic	Microinvasion ≤ 0.1 cm in greatest dimension
T1a	Tumor > 0.1 cm but not < 0.5 cm in greatest dimension
T1b	Tumor > 0.5 cm but not < 1 cm in greatest dimension
T1c	Tumor > 1 cm but not < 2 cm in greatest dimension
T2	Tumor > 2 cm but not > 5 cm in greatest dimension
T3	Tumor > 5 cm in greatest dimension
T4	Tumor of any size, with direct extension to chest wall or skin
T4a	Extension to chest wall
T4b	Edema or ulceration of the skin or satellite skin nodules confined to same breast
T4c	Both T4a and T4b
T4d	Inflammatory carcinoma
Regional Lymph Node (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis to movable ipsilateral axillary lymph node(s)
N2	Metastasis to ipsilateral axillary lymph node(s) fixed to one another or to other structures
N3	Metastasis to ipsilateral internal mammary lymph node(s)
Distant Metastasis (M)	
MX	Distant metastasis cannot be assessed
M0	No distant metastases
M1	Distant metastasis

TABLE 2.2. Breast Cancer Treatments and Their Side Effects[4,5]

Treatment	Description	Side Effects
Surgery		
Mastectomy and axillary node dissection	Removal of entire breast and sampling of lymph nodes	Chest wall pain, reduced arm range of motion, lymphdema
Lumpectomy and axillary node dissection	Removal of breast tumor and sampling of lymph nodes	Reduced arm range of motion, lymphdema
Radiotherapy		
External Beam Radiation	High-energy photons delivered to breast tissue ± locoregional lymph nodes and chest wall	Irreversible lung damage, premature atherosclerosis, cardiomyopathy, lymphedema
Chemotherapy		
Antimetabolic chemotherapy	Methotrexate, Flurouracil, Gemcitabine	Fatigue, anorexia, nausea, anemia, neutropenia, thrombocytopenia
Antitubulin chemotherapy	Taxol, Taxotere, Vinorelbine, Vincristine	Fatigue, muscle pain, ataxia, anemia, neutropenia, thrombocytopenia
Alkylator chemotherapy	Cyclophosphamide	Fatigue, anorexia,nausea, anemia, neutropenia, thrombocytopenia
Anthracycline chemotherapy	Doxorubicin, Epirubicin, Mitoxantrone	Fatigue, cardiotoxicity, nausea, vomiting, anemia, neutropenia, thrombocytopenia, premature menopause
High-dose chemotherapy with bone marrow/stem cell transplantation	Combinations of 2 to 4 chemotherapy drugs in maximal doses	Loss of muscle mass, nausea, vomiting, neuropathy, anemia, neutropenia, thrombocytopenia, infection
Hormone Therapy		
Antiestrogen therapy	Tamoxifen	Weight gain, fatigue, hot flashes, deep venous thrombosis
Aromatase Enzyme Inhibitor therapy	Anastrazole, Letrozole, Exemestane	Fatigue, hot flashes
Progestin therapy	Megesterol	Weight gain, fatigue, dyspnea, hot flashes, deep venous thrombosis
Ovarian Ablation therapy	Ovarian surgery, irradiation, suppression	Weight gain, fatigue, hot flashes, osteoporosis
Glucocorticoid therapy	Dexamethasone, Prednisone	Fat redistribution, osteoporosis, edema, infection

TABLE 2.3. Human Tumor Antigens Associated With the Antitumor Immune Response[14]

Tumor Rejection Antigen	Cancer	Immune System Recognition
Bcr/Abl	Chronic myelogenous leukemia	T cells
Mutated p53	Many cancers	T cells
Immunoglobulin idiotype	B cell lymphoma	Antibodies and T cells
Mutated Ras	Adenocarcinomas	T cells
MAGE 1	Melanoma	T cells
MAGE 3	Melanoma	T cells
MART 1	Melanoma	T cells
Tyrosinase	Melanoma	T cells
Gp100	Melanoma	T cells
MUC 1	Breast, Pancreas	T cells

TABLE 2.4. Cellular Immune System Components Involved in Cancer Defense[6]

Component	Anticancer Function	Mechanism (s) of Action
Cytotoxic T Lymphocyte	Destroy tumor cells	Granule-mediated exocytosis, Fas-Fas ligand interactions
Natural Killer Cells	Destroy tumor cells in absence of MHC I and II antigen expression on target cells	Opposing-Signals Model, Antibody-Dependent Cell-Mediated Cytotoxicity
Macrophages	Destroy tumor cells, antigen presenting, source of interleukin-1 and tumor necrosis factor	Antibody-Dependent Cell-Mediated Cytotoxicity
Neutrophils	Destroy tumor cells	Antibody-Dependent Cell-Mediated Cytotoxicity

TABLE 2.5. Soluble Factors of the Immune System Involved in Cancer Defence[6]

Component	Target Cell	Anticancer Function
Acute Phase Proteins		
C-Reactive Protein	Tumor Cells	Render tumor cells more susceptible to phagocytosis by monocytes
Cytokines		
Interleukin-1	T Cells	Costimulates activation
	B Cells	Promotes maturation and clonal expansion
	Natural Killer Cells	Enhances cytotoxic activity
	Macrophages/Neutrophils	Chemotactically attracts
Tumor Necrosis Factor - α	Tumor Cells	Cytotoxic effects
Tumor Necrosis Factor - β	Tumor Cells	Cytotoxic effects
Interferon - α	Tumor Cells	Inhibits tumor cell growth
	Natural Killer Cells	Activates
	Macrophages	Activates
Interferon - γ	Macrophages	Enhances cytotoxic activity
Oncostatin M	Tumor Cells	Inhibits tumor cell growth

TABLE 2.6. Summary of Studies Examining the Effects of Physical Exercise Training on Immune System Function in Cancer Survivors

Authors	Sample	Design	Physical Exercise Intervention	Immune Component	Immune Assessment Protocol	Results
Physical Exercise During Cancer Treatment						
Dimeo et al.[36]	70 male and female autologous peripheral blood cell transplant patients with solid tumors (39±10y; 40±11y)	Randomized controlled trial with usual care controls	Supervised bed ergometer from first time of high dose chemotherapy until discharge (≈ 2 weeks), 7/week @ 70% intensity, 30 min	Peripheral blood neutrophils collected 12h after the last exercise session	Complete Blood Count	↓ duration of neutropenia and ↓ loss of physical performance after exercise intervention in exercise group compared to control group
Shore et al.[41]	6 children with acute lymphoblastic leukemia or other type of neoplasm (14.0±1y) and 11 healthy controls (10.3± 2y)	Pretest-Posttest with matched controls (3 non-exercising cancer survivors and 11 healthy controls)	Supervised cycling, soccer, skating, cross country skiing, swimming , or combination for 12 weeks, 3-4/week @ 70-85% HRmax, 30 min	Peripheral blood leukocytes, lymphocytes (CD3+, CD4+, CD8+, CD19+, CD25+, CD56+, CD122+) collected 36 h after the last exercise session	Direct Immunofluorescence, Flow Cytometry, ^{51}Cr Release Assay, Mitogen-Stimulated Lymphocyte Proliferation	Baseline: ↓ # leukocytes, CD3+, CD4+, CD8+, CD19+, CD25+, PHA-stimulated lymphocyte proliferation in exercise group cancer survivors receiving chemotherapy compared to healthy controls 12 weeks *: ↓ # CD3+, CD4+, CD8+, CD4+/CD8+, CD25+, ↑ IL-2 stimulated cytolytic activity in exercise group cancer survivors receiving chemotherapy compared to healthy controls; ↑ aerobic power after exercise intervention in exercise group cancer survivors receiving chemotherapy.
Physical Exercise After Cancer Treatment						
Peters et al.[38]	24 female stage I and II breast cancer survivors (49±6.4y)	Pretest-Posttest with no controls	Supervised cycle ergometer for 5 weeks, 5/week @ 60-86% HRmax, 30-40 min post-treatment. Then self-reported cycling for 6 months, 2-3/week @ moderate intensity	Peripheral blood lymphocytes (CD56+)	Direct Immunofluorescence, Flow Cytometry, ^{51}Cr Release Assay	↔ # or % CD56+ at 5 or 29 weeks compared to baseline; ↑ NKCA (% lysis) at 29 weeks compared to baseline and 5 weeks.

Peters et al.[39]	24 female stage I and II breast cancer survivors (49±6.4y)	Pretest-Posttest with no controls	Supervised cycle ergometer for 5 weeks, 5/week @ 60-86% HRmax, 30-40 min post-treatment. Then self-reported cycling for 6 months, 2-3/week @ moderate intensity	Peripheral blood leukocytes, lymphocytes, monocytes, granulocytes	Blood Smear, Functional Test for the Phagocytotic Ability of Monocytes	↔ # leukocytes, ↑ % granulocytes at 29 weeks compared to baseline and 5 weeks, ↓ %, # lymphocytes at 29 weeks compared to 5 weeks, ↓ % monocytes at 29 weeks compared to baseline and 5 weeks; ↑ phagocytosis (%, PI) of monocytes vs. RDSE at 29 weeks compared to baseline, ↔ phagocytosis (%, PI) vs. ADHE at any time point
Nieman et al.[40]	12 female breast cancer survivors (35-72y)	Randomized controlled trial with usual care controls	Supervised walking for 8 weeks, 3/week @ 75% HRmax, 60 min combined with supervised resistance training for 8 weeks, 2 sets of 12 repetitions of 7 exercises	Peripheral blood leukocytes, lymphocytes (CD3+, CD3–CD16+CD56+), neutrophils	Direct Immunofluorescence, Flow Cytometry, ^{51}Cr Release Assay	↔ # of leukocytes, CD3+, CD3-CD16+CD56+, neutrophils, NKCA and ↓ HR during fixed submaximal load, ↑ 16-min walk test distance, ↑ leg strength after exercise intervention in exercise group compared to control group
Na et al.[37]	35 stomach cancer patients (28-75y)	Randomized controlled trial with usual care controls	Supervised range of motion exercise, pelvic tilting exercise, isometric-quadriceps-setting exercise 3/day, 30 min. Then supervised arm or cycle ergometer for 2 weeks, 10/week, 30 min	Peripheral blood lymphocytes	^{51}Cr Release Assay	↑ NKCA (% lysis) after exercise intervention in exercise group compared to control group

Abbreviations and Symbol: y = year; HRmax = maximum heart rate; HR = heart rate; NKCA = natural killer cell cytolytic activity; PI = Phagocytosis Index; RDSE = receptor destroying enzyme-treated sheep erythrocytes; ADHE = anti D-loaded human erythrocytes; ↑ = increase; ↓ = decrease; ↔ = no change; * = non-significant

CHAPTER 3

Effect of Exercise Training on Natural Killer Cell Cytotoxic Activity in Postmenopausal Breast Cancer Survivors: Results From the REHAB (Rehabilitation Exercise for Health After Breast Cancer) Randomized Controlled Trial

3.1 BACKGROUND

Anticancer treatments for postmenopausal breast cancer survivors include surgery, radiotherapy, chemotherapy, and/or hormone therapy.[1,2] Although these treatments have been shown to improve disease-free and overall survival,[3-7] one reported adverse event is impaired immune function.[8-11] Compromised immunity may increase the risk of infection and/or relapse of disease.[12,13] Attenuation of these immunologic effects is desirable.

Exercise training may be one intervention that can improve immune function in cancer survivors.[14-19] Fairey et al. [20] recently performed a systematic review of the literature on this topic. There was preliminary evidence suggesting that exercise training had beneficial effects on natural killer cell cytotoxic activity,[15,16] monocyte function,[17] the proportion of circulating granulocytes,[17] and duration of neutropenia.[14] However, there were several limitations that precluded definitive conclusions from being drawn. These included the sampling procedures, experimental designs, exercise training interventions, and immunologic assessments.[20] Overall, therefore, little is known about the effect of exercise training on immune function in cancer survivors.

The Rehabilitation Exercise for Health After Breast Cancer (REHAB) trial was a randomized controlled trial designed to determine the effects of supervised exercise training on cardiopulmonary, quality of life, and biologic outcomes in postmenopausal breast cancer survivors who had completed therapy. Cardiopulmonary and quality of life outcomes have been reported previously.[21] Significant differences were found favoring the exercise group in oxygen

consumption and power output at peak, oxygen consumption and power output at the ventilatory equivalent for oxygen, and oxygen consumption and power output at the ventilatory equivalent for carbon dioxide. Concomitant significant differences were found for multidimensional quality of life, happiness, fatigue, and self-esteem.

The current thesis reports the effect of exercise training on selected immunologic outcomes. The primary outcome was natural killer cell cytotoxic activity against a natural killer cell sensitive cell line. Secondary outcomes were the percentage of natural killer cells (CD16+CD56+) and white blood cell counts and differentials. The hypothesis was that exercise training would have a beneficial effect on natural killer cell cytotoxic activity.

3.2 METHODS

Details of the setting, participant eligibility criteria, experimental design, recruitment procedures, randomization, blinding, and exercise training intervention have been reported previously.[21] Brief descriptions are provided below.

Setting and Participants

The trial was conducted at the Cross Cancer Institute (CCI) and University of Alberta in Edmonton, Alberta, Canada. The Alberta Cancer Board Research Ethics Committee and the University of Alberta Health Research Ethics Board approved the trial. Written informed consent was obtained for all procedures.

Eligibility criteria included: 1) histologically confirmed early stage breast cancer with no evidence of recurrent or progressive disease, 2) diagnosed between January 1999 and June 2000, 3) completed surgery, radiotherapy, and/or chemotherapy, 4) postmenopausal (not experiencing menstrual periods for the previous 12 months), 5) between 50 to 69 years of age at time of diagnosis, 6) able to understand English, 7) resided in Capital Health Authority region 10, and 8) willing to travel to the exercise facility for exercise training sessions. Eligible participants were not admitted if they had: 1) known cardiac disease, 2) uncontrolled hypertension, 3) unstable thyroid disease, 4) diabetes mellitus, 5) significant mental illness, 6) active infection, 7) immune or endocrine abnormality, and 8) contraindications to exercise as determined by a cardiopulmonary exercise stress test.

Experimental Design and Recruitment

The study was a two group, prospective, randomized controlled trial. A random sample of female breast cancer survivors was obtained using the Alberta Cancer Registry. The referring physician was contacted to provide approval to recruit each survivor. A recruitment letter was mailed to each approved survivor who then contacted the Project Director by telephone if interested. Eligible survivors were mailed a second package that contained a detailed cover letter, two copies of an informed consent, and a questionnaire. These survivors were scheduled for a cardiopulmonary exercise test and blood collection and asked to bring one copy of their completed informed consent and questionnaire to the test.

Randomization

Participants were randomly assigned to the exercise or control group using a random-number table. Participants were stratified by type of adjuvant therapy (chemotherapy versus no chemotherapy and hormone therapy versus no hormone therapy). Stratum 1 included participants who had received chemotherapy and were currently on hormone therapy. Stratum 2 included participants who had received chemotherapy but no hormone therapy. Stratum 3 comprised participants who did not receive chemotherapy but were currently on hormone therapy. Stratum 4 consisted of participants who had not received chemotherapy or hormone therapy. A block permutation procedure was used to generate the allocation sequence within each strata to ensure a close balance of the numbers in each group. The specified allocation ratio was 1:1. Group assignments were enclosed in sequentially numbered and sealed envelopes. A

research assistant generated the allocation sequence and prepared the group assignment envelopes. The envelopes were concealed from the Project Director who assigned participants to groups. The envelopes were opened sequentially after the participant's name was written on the appropriate envelope.

Blinding

Data were collected and analyzed by laboratory staff and investigators who were blinded to treatment assignments.

Exercise Training Intervention

The exercise group trained on nonconsecutive days at the Behavioral Medicine Fitness Center at the University of Alberta. Each participant was assigned to an exercise trainer who supervised every exercise session. Missed sessions were rescheduled at the participant's convenience. Participants trained three times per week for 15 weeks on recumbent or upright cycle ergometers (Lifestyle Fitness 9500HR, Lifecycle Inc). Exercise intensity was based on a cardiopulmonary exercise test at baseline and set at a power output equivalent to the power output at the ventilatory equivalent for carbon dioxide. Levels of intensity during training were monitored via heart rate (Polar Beat Heart Rate Monitor, Polar USA Inc, Stanford, Connecticut), blood pressure (sphygmomanometer), and ratings of perceived exertion. Exercise duration began at 15 minutes for weeks 1 through 3, and then systematically increased by 5 minutes every three weeks thereafter to 35 minutes for weeks 13 through 15. Training sessions included both a warm-up and cool-down period. These periods consisted of 5 minutes of cycling at a power output equivalent to the power

output at the ventilatory equivalent for oxygen followed by a 5-minute regimen of standardized stretches for the lower body.

The control group did not train and were asked not to begin a structured exercise program. To reduce attrition, control group participants were offered the same intervention after a 15-week waiting period.

Outcome Measures

Outcome measures were assessed in peripheral blood. The primary outcome was natural killer cell cytotoxic activity against a natural killer cell sensitive cell line. Secondary outcomes were the percentage of natural killer cells (CD16+CD56+) and white blood cell counts and differentials.

Blood Collection

All blood samples were drawn between 0700 and 1000 with participants in the seated position after a 12-hour fast (including liquids except water). Participants were instructed not to exercise for at least 48 hours prior to blood collection. Participants completed a three-day diet record on consecutive days prior to blood collection at baseline and were requested to consume the same diet for the three days prior to blood collection at postintervention. Twenty-five mL of blood was drawn from each participant in two 10-mL tubes containing sodium heparin and one 5-mL tube containing anticoagulant ethylenediamine tetraacetic acid (EDTA). Samples were chilled on ice during blood collection.

Natural Killer Cell Cytotoxic Activity

Peripheral blood was centrifuged at 700*g* at 23°C for 10 minutes. The red blood cell pellet and buffy coat were re-suspended in 2 mL (1:1 vol/vol) with 10

g/L bovine serum albumin (BSA; Fraction V; Sigma Chemical Co. St. Louis, MO) in phosphate buffered saline (PBS pH=7.4). Mononuclear cells were isolated and purified on a Ficoll density gradient of Histopaque 1077 (Sigma Chemical Co. St. Louis, MO) by centrifugation at 400*g* at 23°C for 30 minutes. The lymphocyte band was recovered on top of the 1077 gradient. Cells were washed with 10 g/L BSA in PBS and spun at 250*g* for 10 minutes and the recovered lymphocyte pellet was diluted to 1 mL with complete RPMI 1640 (defined below) supplemented with 40 g/L heat-inactivated fetal bovine serum (Gibco Life Technologies, Burlington, ON). Cells were counted using a hemocytometer and aliquoted to a cell concentration of 4×10^6 cells/mL for determination of natural killer cell activity. Cell viability was determined using trypan blue exclusion and was greater than 98% for all groups.

Two million human K-562 cells (American Type Tissue Culture Collection, Manassas, VA, USA) were incubated at 37°C for 1 hour with 9.25 MBq/mL sodium chromate labeled chromium (^{51}Cr; Amersham Pharmacia Biotech, Montreal Que). The cells were washed twice and made up to a cell concentration of 0.2 million cells per millilitre and then seeded into a 96-well v-bottom microtiter culture plate. Isolated lymphocytes were added in triplicate to the wells at five effector-to-target ratios varying from 3:1 to 50:1. Following 4 hours incubation at 37°C, a 75uL aliquot of the supernatant was transferred to a Lumaplate and counted on a TopCount NXT (Canberra Packard, Mississauga Ont) to determine ^{51}Cr release. Spontaneous release was determined from the target cells incubated in the absence of effector cells. Maximum release was determined

from detergent lysis of labeled target cells.[22] Natural killer cell activity was calculated as follows: percent specific lysis = 100 × (experimental release – spontaneous release) / (maximum release – spontaneous release).[22] Results were also corrected to a per cell basis using the number of natural killer cells present in the assay. Natural killer cells were considered to be those that expressed both the CD16 and CD56 antigens. This was expressed in lytic units ($LU/10^3$), with LU representing the number of effector cells required to cause 30 percent lysis of target cells.

Percentage of Natural Killer Cells (CD16+CD56+)

Diluted whole blood (100 μL) was added to wells of a 96-well microtitre plate and red blood cells were lysed using warm lysis buffer (155 mol/L ammonium chloride, 20 mol/L sodium bicarbonate and 0.5 mol/L EDTA). Natural killer cells were then identified by an immunofluorescence assay as previously described.[22] The following anti-human mouse monoclonal antibodies (mAbs; Sigma Chemical Co. St. Louis, MO, USA and BD Pharmingen, Mississauga, ON) labelled with FITC (Fluorescein isothiocyanate) or B (Biotin) were used: CD16-FITC (NK cells and macrophages) and CD56-B (NK cells). Leukocytes from whole blood (depleted of red blood cells) were incubated at 4°C for 30 minutes with mAbs and washed once in 200 μL of PBS containing fetal bovine serum (40 g/L). Wells containing biotin-labelled mAb against the CD56 antigenic marker were incubated at 4°C for another 30 minutes with the streptavidin conjugated QR (Sigma Chemical Co, St. Louis, MO). Cells were washed and fixed in paraformaldehyde (10 g/L in PBS with sodium azide as a perservative) and all

samples were acquired on the same flow cytometer (FACScan™; Becton Dickinson, Sunnyvale, CA) according to relative fluorescence intensity within 3 days. Analysis (10,000 cells per mAb combination) was performed on the gated lymphocyte population, which was set to exclude any remaining nucleated red blood cells. Appropriate isotype controls (Sigma Chemical Co, St. Louis, MO) were used for each labeled mAb and resultant percentages were corrected for background fluorescence (< 1 percent).

White Blood Cell Counts and Differentials

White blood cell counts and differentials were measured using a Coulter STKS instrument (Coulter Electronics, Inc., Hialeah, FA) by staff at the Dynacare Kaspers Laboratory in Edmonton, Alberta, Canada.

Baseline Characteristics, Blood Collection Protocol Information, and Adverse Events

Baseline characteristics included demographic, medical, and past exercise data. Demographic data was collected using self-report scales and medical variables were abstracted from medical records. Past exercise and non-protocol related exercise performed during the study was assessed by the leisure score index of the Godin Leisure-Time Exercise Questionnaire.[24] Blood collection protocol information included daily medication use, blood transfusions during the intervention period, and sleeping patterns and nutrient intake prior to blood collection. All data was collected by self-report. Nutrient intake was determined from diet records that were analyzed using the Food Processor II™ program (ESHA Research, Oregon). Analyzed records provided data on energy intake

and nutrient sources. Three-day averages for total energy, carbohydrate, fat, and protein were calculated (g and percent of total daily energy intake). All adverse events reported by the participant or observed by the investigator were recorded. An adverse event was defined as any adverse change from the participant's baseline condition, whether it was considered related to exercise training or not.

Sample Size Calculation and Statistical Analysis

Sample size calculation was based on the primary cardiopulmonary and quality of life outcomes for the REHAB trial.[21] To detect a large standardized effect (d=.80) with a power of .80 and a two-tailed alpha less than .05 significance level, 25 participants were required in each group.[25] Fifty-three participants were enrolled to compensate for those who were non-evaluable. The population for our pre-specified intention-to-treat analysis was defined as the participants who were randomly assigned to treatment and for whom the data was sufficient to permit evaluation. All analyses were performed using SPSS version 10.0 for Windows package (SPSS, Inc., Evanston, Illinois).

Baseline characteristics, adverse events, and blood collection protocol information of the two groups were compared using independent-samples t-tests for continuous data and Pearson's chi-square tests for categorical data. Change score analyses utilized independent-samples t-tests to compare changes between groups in outcome measures from baseline to postintervention. Change over the treatment period was calculated by subtracting the baseline score from the postintervention score. Between group analyses used independent-samples t-tests to compare differences between groups at baseline and postintervention.

Within group analyses utilized paired-samples t-tests to compare changes within groups from baseline to postintervention. Data are presented as the mean (standard deviation) with 95% confidence intervals.

3.3 RESULTS

Flow of Participants Through the Trial

Participant recruitment took place in May and June 2001. Baseline assessments were completed in July 2001 and postintervention assessments were conducted in November 2001. A total of 370 breast cancer survivors were assessed for eligibility during the recruitment period, and 53 (14.3%) were randomized. Twenty-five participants were assigned to the exercise group and 28 to the control group. During the intervention period, 1 participant (4.2%) was excluded from further analyses in the exercise group compared to 0 participants in the control group (p=.285). The participant in the exercise group was excluded because of a gastrointestinal complication unrelated to exercise (n=1). Overall, 52 out of 53 participants completed the trial (98.1%).

Baseline Characteristics, Blood Collection Protocol Information, and Adverse Events

Baseline characteristics and blood collection protocol information is shown in Tables 3.1 and 3.2, respectively. No significant differences were observed between groups for any variable. During the intervention period, five participants (20.8%) in the exercise group experienced an adverse event compared to two participants (7.1%) in the control group (p=.168). Adverse events in the exercise group included lymphedema (n=3), gynecologic complication (n=1), and influenza (n=1). Adverse events in the control group included foot fracture (n=1) and bronchitis (n=1).

Adherence to the Exercise Training Intervention

Details of adherence to the exercise training intervention have been reported previously.[21] In brief, the exercise group completed an average of 44.3 out of 45 exercise training sessions (98.4%). Nonprotocol-related exercise was low and not significantly different between groups (p=.890). The exercise group reported an average of 15 minutes of moderate/strenuous nonprotocol-related exercise per week compared to 13 minutes in the control group.

Natural Killer Cell Cytotoxic Activity

Change score analyses of percent specific lysis of ^{51}Cr-labelled tumour cells and total lytic units are shown in Table 3.3. In the exercise group, percent specific lysis at the 3.125:1 effector-to-target ratio increased by 5.2 percent whereas it decreased by 0.1 percent in the control group (mean difference, –5.3 percent; 95% CI, –9.4 to 1.3 percent; p=.011). Concomitant borderline significant interactions for percent specific lysis were observed at both the 25:1 (mean difference, –6.9 percent; 95% CI, –14.8 to 1.1 percent; p=.088) and 50:1 (mean difference, –7.5 percent; 95% CI, –16.0 to 1.2 percent; p=.090) effector-to-target ratios.

Between group analyses of percent specific lysis of ^{51}Cr-labelled tumour cells and total lytic units are shown in Table 3.4. Baseline values were not significantly different between groups. At postintervention, the exercise group showed significantly higher percent specific lysis at the 3.125:1 (mean difference, +6.7 percent; 95% CI, 3.5 to 10.0 percent; p<.001), 6.25:1 (mean difference, +7.8 percent; 95% CI, 2.2 to 13.5 percent; p=.007), 12.5:1 (mean difference, +7.5 percent; 95% CI, 1.8 to 13.2 percent; p=.011), and 25:1 (mean difference, +5.8

percent, 95% CI, 0.1 to 11.4 percent; p=.045) effector-to-target ratios, and higher lytic activity per cell (mean difference, –3.08 LU; 95% CI, –5.85 to –0.22 LU; p=.035) compared to the control group.

Within group analyses of percent specific lysis of ^{51}Cr-labelled tumour cells and total lytic units are shown in Table 3.5. The exercise group had a significant increase in percent specific lysis at the 3.125:1 (mean change, +5.2 percent; 95% CI, 2.0 to 8.4 percent; p=.003), 6.25:1 (mean change, +6.0 percent; 95% CI, 0.9 to 11.0 percent; p=.022), and 12.5:1 (mean change, +5.1 percent; 95% CI, 0.3 to 9.8 percent; p=.037) effector-to-target ratios, and a borderline significant increase in lytic activity per cell (mean change, –3.38 LU; 95% CI, –6.82 to 0.06 LU; p=.054) whereas there were no changes in the control group.

Percentage of Natural Killer Cells (CD16+CD56+)

Change score analyses of the percentage of natural killer cells (CD16+CD56+) showed no significant difference in the change score between groups from baseline to postintervention (mean difference, 0 percent; 95% CI, –3 to 2 percent; p=.652). Between group analyses showed no significant difference between groups at baseline (mean difference, 0 percent; 95% CI, –3 to 4 percent; p=.929) or postintervention (mean difference, –1 percent; 95% CI, –3 to 2 percent; p=.737). Within group analyses showed no significant change from baseline to postintervention in the exercise group (mean change, +1 percent; 95% CI, –2 to 2 percent; p=.858) or control group (mean change, 0 percent; 95% CI, –2 to 2 percent; p=.652).

White Blood Cell Counts and Differentials

Change score analyses for white blood cell counts and differentials are shown in Table 3.6. In the exercise group, the percentage of neutrophils decreased by 2 percent whereas it increased by 3 percent in the control group (mean difference, +4.8 percent; 95% CI, 0.2 to 9.3 percent; p=.042). No other significant differences in change scores between groups from baseline to postintervention were observed.

Between group analyses for white blood cell counts and differentials are shown in Table 3.7. Baseline values were not significantly different between groups except that the exercise group had a significantly higher absolute number of neutrophils (mean difference, $+0.7\times10^9$ cells/L; 95% CI, 0.05×10^9 to 1.4×10^9 cells/L; p=.036). No significant differences between groups were found at postintervention.

Within group analyses for white blood cell counts and differentials are shown in Table 3.8. No significant changes were found between baseline and postintervention in either group.

3.4 DISCUSSION

This randomized controlled trial examined the effect of exercise training on natural killer cell cytotoxic activity in postmenopausal breast cancer survivors who had completed therapy. Secondary outcomes were the percentage of natural killer cells and white blood cell counts and differentials. It was found that supervised exercise training had a beneficial effect on natural killer cell cytotoxic activity.

These results support the hypothesis that exercise training can have a beneficial effect on natural killer cell cytotoxic activity in breast cancer survivors. Percent specific lysis over the five effector-to-target ratios increased by an average of 27.4 percent in the exercise group whereas it did not change in the control group. The results of this study extend previous data showing that exercise training interventions can have beneficial effect on natural killer cell cytotoxic activity in cancer survivors.[15,16] The magnitude of the treatment effect in percent specific lysis associated with the exercise training intervention in this trial was higher than that reported in previous trials. In a nonrandomized trial, Peters et al.[16] reported that the percent specific lysis increased by 9.4 percent in breast cancer survivors assigned to 29 weeks of cycle ergometer exercise. In a randomized trial, Na et al.[15] reported that percent specific lysis increased by 11.7 percent in stomach cancer survivors assigned to 2 weeks of combined modality exercise training whereas it decreased by 6.4 percent in the control group. Reasons for the discrepant findings in magnitude of the treatment effect remain to be determined. Possible explanations include the parameters of the exercise

training intervention (i.e., frequency, intensity, time, modality), timing of the intervention in relation to treatment, and method used to assess percent specific lysis.

Previous studies in cancer survivors have not demonstrated that exercise training can have a beneficial effect on the cytotoxic activity per cell.[20] Reasons for this remain to be determined. In contrast, exercise training was effective in the current trial. Per cell cytotoxic activity was not different between groups at baseline; however, the exercise group had higher per cell cytotoxic activity (as evidenced by a decrease in total lytic units) compared to the control group at postintervention. This finding is consistent with the observation that exercise training can improve natural killer cell cytotoxic activity beyond that which is associated with normal recovery after anticancer therapy.[26]

Exercise training may have had a beneficial effect on natural killer cell activity by a variety of mechanisms. Some of these include neuroendocrinologic factors (catecholamines, growth hormone, cortisol, β-endorphin, sex steroids), metabolic factors (glutamine, glucose, lipids, antioxidants), and other immunologic factors (interferon-α, interleukin-2).[27] Importantly, the changes were unrelated to demographic and medical variables, past exercise habits, medication use, macronutrient intake, blood transfusions, sleeping patterns, weight loss, or smoking cessation during the intervention period.

An important consideration is whether natural killer cells can influence clinical outcomes. The importance of natural killer cells in anti-tumour immunity has recently been demonstrated in natural killer cell-deficient mice defined during

the generation of LY-49A transgenic mice.[28] In this study, a founder and its offspring displayed reduced levels of NK1.1+CD3– cells, lacked detectable *in vitro* natural killer cell activity, and had impaired ability to control tumour growth and metastasis *in vivo*. Although natural killer cells are known to have a pre-eminent role in the destruction of virus-infected and malignant cells,[29] their impact on important clinical outcomes such as infection and/or relapse of disease in human subjects remains to be determined.

Participation and adherence rates in this trial should be noted. Postmenopausal breast cancer survivors were interested in the exercise training intervention. The participation rate of eligible women was 14.3 percent. This compares favourably to both chemotherapy and radiotherapy trials, which report participation rates between 3 and 12 percent.[30-32] The participation rate is also higher than the 8.6 percent rate that was recently reported for a trial of psychosocial group support.[33] Participants were also committed to the exercise training intervention. Women in the exercise group attended 98.4 percent of their prescribed exercise training sessions. This is much higher than previous trials. For example, Segal et al.[34] reported a 70 percent adherence rate to an exercise training intervention in 99 early stage breast cancer survivors receiving adjuvant therapy. Reasons for the high rate of adherence in this trial may include the strictly controlled exercise training intervention, safe and supervised exercise facility, free parking, close monitoring of attendance, and staff at the exercise laboratory.

Rates of adverse events were similar between groups. Moreover, the exercise training intervention was well tolerated. Although exhaustive exercise training can suppress immune function,[35] there was no evidence of worsening immunologic status. This is probably related to the moderate intensity exercise training that was prescribed in this trial. Overall, therefore, the exercise training intervention was both safe and effective.

This study had several limitations. First, the study sample was small. Therefore, the findings should be confirmed in trials with larger cohorts. The small sample size also limited the ability to perform subgroup analyses, such as for women with lower natural killer cell cytotoxic activity at baseline. Second, the exercise training intervention was only 15 weeks in length and no long-term follow-up was provided. Consequently, the findings are restricted to the short-term effects of exercise training in breast cancer survivors. Third, changes in immune function were measured in blood, which constitutes approximately 10 percent of the total lymphoid cell population.[36] Therefore, it is unknown whether these adaptations are representative of the condition of the whole body. Importantly, however, it was not feasible to obtain these cells from biopsy specimens.

In summary, the REHAB trial showed that exercise training had a beneficial effect on natural killer cell cytotoxic activity in postmenopausal breast cancer survivors who had completed therapy. It also demonstrated that this population was interested in and committed to a short-term exercise training intervention. Further research is needed to determine the impact of increased

. natural killer cell cytotoxic activity on important clinical outcomes such as risk of infection and/or relapse of disease.

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Table 3.1. Baseline Characteristics ‡

Variable	Overall (n=52)	Exercise Group (n=24)	Control Group (n=28)	P Value†
Demographic				
Age (years)	59 (6)	59 (5)	58 (6)	.712
Married	39 (75%)	18 (75%)	21 (75%)	1.000
Completed University	23 (44%)	7 (29%)	16 (56%)	.058
Family Income > $60,000/year	23 (44%)	10 (44%)	13 (48%)	.741
Employed Full-Time	15 (29%)	7 (29%)	8 (29%)	.962
Medical				
Weight (kg)	78.7 (18.1)	78.1 (20.4)	79.4 (16.4)	.801
Body Mass Index (kg/m^2)	29.2 (6.6)	29.4 (7.4)	29.1 (6.1)	.880
Sum of Skinfolds (mm)	134.3 (40.5)	136.8 (45.1)	132.1 (36.5)	.682
Months Posttreatment	14 (6)	14 (6)	14 (7)	.856
Tumor Stage				
T1	28 (54%)	12 (50%)	16 (57%)	.797
T2	18 (35%)	8 (33%)	10 (36%)	.857
T3	6 (12%)	4 (17%)	2 (7%)	.284
TX	11 (2%)	0 (0%)	1 (3.6%)	.350
Nodal Stage				
N0	35 (67%)	16 (67%)	19 (68%)	.927
N1	16 (31%)	7 (29%)	9 (32%)	.817
N2	1 (2%)	1 (4%)	0 (0%)	.275
Surgery				
Mastectomy	28 (54%)	15 (63%)	13 (46%)	.246
Lumpectomy	24 (46%)	9 (37%)	15 (54%)	.246
Locoregional Radiotherapy	37 (71%)	16 (67%)	21 (75%)	.508
Adjuvant Chemotherapy	21 (40%)	10 (42%)	11 (39%)	.862
Adjuvant Hormone Therapy	24 (46%)	11 (46%)	13 (46%)	.966
Past Exercise				
Moderate minutes	82 (114)	62 (94)	98 (126)	.247
Strenuous minutes	25 (61)	23 (56)	26 (65)	.897
Moderate/Strenuous minutes	106 (129)	85 (102)	124 (146)	.280
Percent >90 Moderate/Strenuous	22 (42%)	10 (42%)	12 (42.9%)	.931

‡ Data are presented as the mean (standard deviation) for continuous variables and frequency (percentage) for categorical variables.
† P value for the difference between groups at baseline.

Table 3.2. Blood Collection Protocol Information ‡

Variable	Overall (n=52)	Exercise Group (n = 24)	Control Group (n = 28)	P Value †
Medication Use				
ACE Inhibitor	3 (5%)	1 (4%)	2 (7%)	.646
Acetaminophen	3 (5%)	3 (13%)	0 (0%)	.054
α-Antagonist	2 (4%)	2 (8%)	0 (0%)	.119
Antibiotic	1 (2%)	0 (0%)	1 (4%)	.350
Anti-fungal	1 (2%)	0 (0%)	1 (4%)	.350
Anti-inflammatory	15 (29%)	7 (29%)	8 (29%)	.962
Aspirin	3 (5%)	2 (8%)	1 (4%)	.463
Atorvastatin	3 (5%)	1 (4%)	2 (7%)	.646
β-Blocker	1 (2%)	0 (0%)	1 (4%)	.350
Bisphosphonate	11 (19%)	7 (29%)	4 (14%)	.190
Calcium Channel Blocker	1 (2%)	1 (4%)	1 (4%)	..911
Diuretic	2 (4%)	1 (4%)	1 (4%)	.911
Hormone Replacement Therapy	1 (2%)	0 (0%)	1 (4%)	.350
Hydrogen Ion Blocker	1 (2%)	0 (0%)	1 (4%)	.350
Leukotriene Antagonist	1 (2%)	1 (4%)	0 (0%)	.275
Nasal Steroid	2 (4%)	1 (4%)	1 (4%)	.911
Proton Pump Inhibitor	4 (7%)	3 (13%)	1 (4%)	.228
Sedative	6 (12%)	4 (17%)	2 (7%)	.284
Selective Serotonin Reuptake Inhibitor	11 (19%)	6 (25%)	5 (18%)	.530
Sumatriptan	1 (2%)	1 (4%)	0 (0%)	.275
Thyroid Hormone	13 (23%)	3 (13%)	10 (36%)	.054
Ventolin	1 (2%)	0 (0%)	1 (4%)	.350
Nutrient Intake				
Total Calories (Kcal/day)	1780 (389)	1782 (391)	1779 (395)	.979
Percent Calories from Fat	33 (6)	34 (5)	32 (7)	.288
Percent Calories from Carbohydrates	50 (8)	49 (6)	51 (8)	.253
Percent Calories from Protein	16 (3)	16 (3)	16 (4)	.440
Total Fat (g)	590 (154)	604 (140)	578 (167)	.541
Total Carbohydrate (g)	228 (61)	220 (54)	234 (67)	.428
Total Protein (g)	73 (23)	74 (21)	71 (25)	.663
Miscellaneous Blood Draw Information				
Blood Transfusions	0 (0)	0 (0)	0 (0)	1.000
Hours Sleep Prior to Blood Draw at Baseline	7 (1)	7 (1)	7 (1)	.898
Hours Sleep Prior to Blood Draw at Postintervention	7 (1)	7 (1)	7 (1)	.506

‡ Data are presented as the mean (standard deviation) for continuous variables and frequency (percentage) for categorical variables.
† P value for the difference between groups at baseline.

Table 3.3. Change Scores Analyses for the Effects of Exercise Training on Natural Killer Cell Cytotoxic Activity

Variable	Baseline	Postintervention	Mean Change	Difference Between Groups in Mean Change [95% CI]	P Value †
Natural Killer Cell Cytotoxic Activity, effector-to-target ratios % lysis					
50:1					
Exercise Group (n = 24)	55.5 (12.1)	61.4 (9.8)	+5.9 (14.6)		
Control Group (n = 27)	58.0 (12.9)	56.4 (10.5)	−1.6 (15.9)	−7.5 [−16.0 to 1.2]	.090
25:1					
Exercise Group (n = 24)	44.2 (12.8)	49.8 (8.3)	+5.6 (14.4)		
Control Group (n = 27)	45.3 (12.1)	44.0 (11.3)	−1.3 (13.9)	−6.9 [−14.8 to 1.1]	.088
12.5:1					
Exercise Group (n = 22)	36.2 (10.6)	41.2 (8.4)	+5.1 (10.6)		
Control Group (n = 25)	32.2 (10.1)	33.8 (10.7)	+1.5 (13.0)	−3.6 [−10.6 to 3.5]	.319
6.25:1					
Exercise Group (n = 22)	21.7 (9.0)	27.7 (10.1)	+6.0 (11.4)		
Control Group (n = 26)	18.9 (9.4)	19.8 (9.2)	+.9 (13.1)	−5.1 [−12.3 to 2.1]	.161
3.125:1					
Exercise Group (n = 23)	7.2 (5.1)	12.4 (6.6)	+5.2 (7.4)		
Control Group (n = 26)	5.8 (4.5)	5.7 (4.2)	−.1 (6.8)	−5.3 [−9.4 to −1.3]	.011
Total Lytic Units					
Exercise Group (n = 23)	11.98 (6.76)	8.60 (3.40)	−3.38 (7.97)		
Control Group (n = 26)	12.72 (8.19)	11.68 (6.00)	−1.04 (6.44)	2.34 [−1.80 to 6.49]	.261

† P value for the difference between groups in mean change from baseline to postintervention.

Table 3.4. Between Group Analyses for the Effects of Exercise Training on Natural Killer Cell Cytotoxic Activity

Variable	Exercise Group	Control Group	Difference Between Groups [95%CI]	P Value †
Natural Killer Cell Cytotoxic Activity, effector-to-target ratios % lysis				
50:1				
Baseline	55.5 (12.1)	58.0 (12.9)	−2.5 [−9.5 to 4.6]	.488
Postintervention	61.4 (9.8)	56.4 (10.5)	+5.0 [−.76 to 10.7]	.088
25:1				
Baseline	44.2 (12.8)	45.3 (12.1)	−1.1 [−8.1 to 5.9]	.747
Postintervention	49.8 (8.3)	44.0 (11.3)	+5.8 [.1 to 11.4]	.045
12.5:1				
Baseline	36.2 (10.6)	32.2 (10.1)	+3.9 [−2.1 to 10.0]	.198
Postintervention	41.2 (8.4)	33.8 (10.7)	+7.5 [1.8 to 13.2]	.045
6.25:1				
Baseline	21.7 (9.0)	18.9 (9.4)	+2.8 [−2.6 to 8.1]	.305
Postintervention	27.7 (10.1)	19.8 (9.2)	+7.8 [2.2 to 13.5]	.007
3.125:1				
Baseline	7.2 (5.1)	5.8 (4.5)	+1.4 [3.5 to 10.0]	.307
Postintervention	12.4 (6.6	5.7 (4.2)	+6.7 [3.5 to 10.0]	<.001
Total Lytic Units				
Baseline	11.98 (6.76)	12.72 (8.19)	−.73 [−5.08 to 3.62]	.736
Postintervention	8.60 (3.40)	11.68 (6.00)	−3.08 [−5.85 to −.22]	.035

† P value for the difference between groups at baseline and postintervention

Table 3.5. Within Group Analyses for the Effects of Exercise Training on Natural Killer Cell Cytotoxic Activity

Variable	Baseline	Postintervention	Mean Change	% Change	95% CI ††	P Value †
Natural Killer Cell Cytotoxic Activity, effector-to-target ratios % lysis						
50:1						
Exercise Group (n = 24)	55.5 (12.1)	61.4 (9.8)	+5.9 (14.6)	+10.6	–.3 to 12.0	.063
Control Group (n = 27)	58.0 (12.9)	56.4 (10.5)	–1.6 (15.9)	–2.8	–7.9 to 4.7	.606
25:1						
Exercise Group (n = 24)	44.2 (12.8)	49.8 (8.3)	+5.6 (14.4)	+12.7	–.45 to 11.7	.068
Control Group (n = 27)	45.3 (12.1)	44.0 (11.3)	–1.3 (13.9)	–2.9	–6.7 to 4.2	.640
12.5:1						
Exercise Group (n = 22)	36.2 (10.6)	41.2 (8.4)	+5.1 (10.6)	+14.1	.3 to 9.8	.037
Control Group (n = 25)	32.2 (10.1)	33.8 (10.7)	+1.5 (13.0)	+4.7	–3.9 to 6.9	.565
6.25:1						
Exercise Group (n = 22)	21.7 (9.0)	27.7 (10.1)	+6.0 (11.4)	+27.6	.9 to 11.0	.022
Control Group (n = 26)	18.9 (9.4)	19.8 (9.2)	+.9 (13.1)	+4.8	–4.4 to 6.2	.728
3.125:1						
Exercise Group (n = 23)	7.2 (5.1)	12.4 (6.6)	+5.2 (7.4)	+72.2	2.0 to 8.4	.003
Control Group (n = 26)	5.8 (4.5)	5.7 (4.2)	–.1 (6.8)	–1.7	–2.9 to 2.6	.925
Total Lytic Units						
Exercise Group (n = 23)	11.98 (6.76)	8.60 (3.40)	–3.38 (7.97)	–28.2	–6.82 to .06	.054
Control Group (n = 26)	12.72 (8.19)	11.68 (6.00)	–1.04 (6.44)	–8.2	–3.64 to 1.57	.420

† P value for the mean change from baseline to postintervention within each group.
†† 95% CIs are reported for the difference between groups in mean change from baseline to postintervention (Control Group – Exercise Group).

Table 3.6. Change Scores Analysis for the Effects of Exercise Training on White Blood Cell Counts and Differentials

Variable	Baseline	Postintervention	Mean Change	Difference Between Groups in Mean Change [95% CI] ††	P Value †
White Blood Cells ($\times 10^9$/L)					
Exercise Group (n = 24)	5.3 (1.9)	5.0 (1.4)	−.3 (1.4)		
Control Group (n = 28)	4.6 (1.3)	4.7 (1.3)	+.1 (.1)	+.4 [−.2 to .9]	.224
Neutrophils ($\times 10^9$/L)					
Exercise Group (n = 24)	3.2 (1.5)	2.8 (1.1)	−.4 (1.2)		
Control Group (n = 28)	2.5 (.8)	2.6 (.8)	+.1 (.7)	+.5 [−.1 to 1.0]	.076
Lymphocytes ($\times 10^9$/L)					
Exercise Group (n = 24)	1.5 (.6)	1.5 (.5)	0 (.3)		
Control Group (n = 28)	1.5 (.6)	1.5 (.6)	0 (.2)	0 [−.2 to .1]	.865
Monocytes ($\times 10^9$/L)					
Exercise Group (n = 24)	.4 (.2)	.4 (.1)	0 (.1)		
Control Group (n = 28)	.4 (.1)	.4 (.1)	0 (0)	0 [−.05 to .07]	.774
Neutrophils (%)					
Exercise Group (n = 23)	59 (10)	57 (9)	−2 (6)		
Control Group (n = 28)	54 (8)	57 (8)	+3 (9)	+4.8 [.2 to 9.3]	.042
Lymphocytes (%)					
Exercise Group (n = 24)	30 (9)	32 (8)	+2 (5)		
Control Group (n = 28)	33 (7)	32 (7)	−1 (5)	−2.2 [−5.0 to .7]	.134
Monocytes (%)					
Exercise Group (n = 24)	7 (2)	8 (3)	+1 (2)		
Control Group (n = 28)	8 (2)	8 (2)	0 (1)	−.6 [−1.4 to .3]	.149

†† 95% CIs are reported for the difference between groups in mean change from baseline to postintervention (Control Group – Exercise Group).
† P value for the difference between groups in mean change from baseline to postintervention (Control Group – Exercise Group).

Table 3.7. Between Group Analyses for the Effects of Exercise Training on White Blood Cell Counts and Differentials

Variable	Exercise Group	Control Group	Difference Between Groups [95%CI]	P Value †
White Blood Cells ($\times 10^9$/L)				
Baseline	5.3 (1.9)	4.6 (1.3)	+.7 [−1.6 to .2]	.143
Postintervention	5.0 (1.4)	4.7 (1.3)	+.3 [−.4 to 1.0]	.415
Neutrophils ($\times 10^9$/L)				
Baseline	3.2 (1.5)	2.5 (.8)	+.7 [.05 to 1.4]	.036
Postintervention	2.8 (1.1)	2.6 (.8)	+.2 [−.3 to .8]	.366
Lymphocytes ($\times 10^9$/L)				
Baseline	1.5 (.6)	1.5 (.6)	0 [−.3 to .3]	.967
Postintervention	1.5 (.5)	1.5 (.6)	0 [−.3 to .3]	.897
Monocytes ($\times 10^9$/L)				
Baseline	.4 (.2)	.4 (.1)	0 [−.05 to .1]	.397
Postintervention	.4 (.1)	.4 (.1)	0 [−.05 to .1]	.485
Neutrophils (%)				
Baseline	59 (10)	54 (8)	+5 [0 to 11]	.054
Postintervention	57 (9)	57 (8)	0 [−4 to 5]	.834
Lymphocytes (%)				
Baseline	30 (9)	33 (7)	−3 [−7 to 2]	.262
Postintervention	32 (8)	32 (7)	0 [−5 to 4]	.821
Monocytes (%)				
Baseline	7 (2)	8 (2)	−1 [−1 to 1]	.456
Postintervention	8 (3)	8 (2)	0 [−1 to 1]	.721

† P value for the difference between groups at baseline and postintervention

Table 3.8. Within Group Analyses for the Effects of Exercise Training on White Blood Cell Counts and Differentials

Variable	Baseline	Postintervention	Mean Change	% Change	95% CI ††	P Value †
White Blood Cells ($\times 10^9$/L)						
Exercise Group (n=24)	5.3 (1.9)	5.0 (1.4)	−.3	−5.7	−.3 to .9	.297
Control Group (n=28)	4.6 (1.3)	4.7 (1.3)	+.1	+2.2	−.3 to .2	.635
Neutrophils ($\times 10^9$/L)						
Exercise Group (n=24)	3.2 (1.5)	2.8 (1.1)	−.4	−12.5	−.2 to .8	.198
Control Group (n=28)	2.5 (.8)	2.6 (.8)	+.1	+4.0	−.4 to .1	.232
Lymphocytes ($\times 10^9$/L)						
Exercise Group (n=24)	1.5 (.6)	1.5 (.5)	0	0	−.1 to .1	.845
Control Group (n=28)	1.5 (.6)	1.5 (.6)	0	0	−.1 to .1	1.000
Monocytes ($\times 10^9$/L)						
Exercise Group (n=24)	.4 (.2)	.4 (.1)	0	0	−.1 to .1	.759
Control Group (n=28)	.4 (.1)	.4 (.1)	0	0	−.03 to .03	1.000
Neutrophils ($\times 10^9$/L)						
Exercise Group (n=24)	59 (10)	57 (9)	−2	−3.4	−.4 to 5.0	.097
Control Group (n=28)	54 (8)	57 (8)	+3	+5.6	−6.2 to 1.2	.173
Lymphocytes ($\times 10^9$/L)						
Exercise Group (n=24)	30 (9)	32 (8)	+2	+6.7	−3.7 to .7	.164
Control Group (n=28)	33 (7)	32 (7)	−1	−3.0	−1.3 to 2.4	.534
Monocytes ($\times 10^9$/L)						
Exercise Group (n=24)	7 (2)	8 (3)	+1	+14.2	−1.1 to .4	.363
Control Group (n=28)	8 (2)	8 (2)	0	0	−.2 to .7	.229

† P value for the mean change from baseline to postintervention within each group.

†† 95% CIs are reported for the difference between groups in mean change from baseline to postintervention (Control Group – Exercise Group).

CHAPTER 4

General Discussion

4.1 Key Finding

In this randomized controlled trial, it was found that supervised exercise training had a beneficial effect on natural killer cell cytotoxic activity in postmenopausal breast cancer survivors who had completed therapy. The results of this trial extend the results from previous trials in cancer survivors.[1,2]

4.2 Strengths and Limitations of the Trial Design

Certain strengths of the design of this trial should be noted. First, the method used to assign trial participants to treatment groups was random assignment. Randomization had several advantages including the fact that it removed bias in treatment assignment, prevented selection and confounding bias, and permitted the identity of treatment assignment to be blinded to the individuals who assessed outcome measures.[3,4] Second, this trial had appropriate statistical power. Post hoc power calculations suggested that the sample provided 90 percent power to detect a 20 percent difference between groups in natural killer cell cytotoxic activity (standard deviation = 20 percent) based on a formula for sample size calculations for continuous response variables.[5] Third, the exercise training intervention was supervised and strictly controlled.

Certain limitations of the design of this trial should also be noted. First, the trial was not designed to test underlying mechanisms mediating an association between exercise training and natural killer cell cytotoxic activity. Possible mechanisms include neuroendocrinologic factors (catecholamines, growth hormone, cortisol, β-endorphin, sex steroid hormones), metabolic factors

(glutamine, glucose, lipids, antioxidants), and other immunologic factors (interferon-α, interleukin-2).[6] However, several ancillary analyses are planned, which may help to elucidate directions for future mechanistic studies. These studies include the assessment of other immunologic outcomes (lymphocyte proliferation, cytokine production), metabolic hormones outcomes (glucose, insulin, IGF-1, IGFBP-3), sex steroid hormones outcomes (estradiol, estrone, testosterone, androstenedione, dehydroepianodrosterone, dehydroepianodrosterone sulfate, sex hormone binding globulin), and lipid outcomes (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides). Second, this trial was not designed to determine the effect of natural killer cell cytotoxic activity on important clinical outcomes such as risk of infection and/or relapse of disease. Future randomized trials, using clinically important endpoints as the primary outcome, are needed to address this important question.

4.3 Directions for Future Research

Several directions for future research have been identified. Randomized trials of exercise training and natural killer cell cytotoxic activity should be extended to breast cancer survivors of diverse ethnic, racial, and age profiles. It should also be repeated in survivors at particularly high risk for recurrent disease. Other trial designs will also provide important data. Such designs may alter the exercise training parameters to examine both resistance and aerobic exercise interventions. They may also modify the frequency, intensity, and duration of the intervention.

4.4 Long-Term Exercise Adherence

The benefits of exercise can only be attained through regular participation. Rates of physical exercise among cancer survivors are low and long-term adherence to exercise interventions is a difficult challenge. Indeed, research has shown that physical exercise levels decline during cancer treatments and may not return to pre-diagnosis levels following completion of therapy.[7] Interventions designed to improve exercise adherence are therefore needed. One intervention that may be beneficial in this regard is an oncologists' recommendation to exercise. Results from the first randomized trial to examine this question are pending.[8]

4.5 Summary

The REHAB trial was a randomized controlled trial designed to determine the effects of supervised exercise training on cardiopulmonary, quality of life, and biologic outcomes in postmenopausal breast cancer survivors who had completed therapy. The current thesis reported the effect on selected immunologic outcomes. The primary outcome was natural killer cell cytotoxic activity. Secondary outcomes were the percentage of natural killer cells and white blood cell counts and differentials. It was determined that exercise training had a beneficial effect on natural killer cell cytotoxic activity. The experience gained from this trial should be used in the design of larger randomized trials. Such trials are likely to provide information about the utility and feasibility of exercise training as a health outcome intervention for breast cancer survivors.

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APPENDIX

5.1 REFERENCE RANGES FOR WHITE BLOOD CELL COUNTS AND DIFFERENTIALS

Parameter	Reference Range
Counts	
White Blood Cell Counts ($\times 10^9$/L)	4.0 to 11.0
Neutrophils ($\times 10^9$/L)	1.8 to 7.5
Lymphocytes ($\times 10^9$/L)	1.0 to 4.5
Monocytes ($\times 10^9$/L)	0.0 to 0.1
Differentials	
Neutrophils (%)	See above
Lymphocytes (%)	See above
Monocytes (%)	See above

Note: Reference ranges were obtained from Dynacare Kasper Medical Laboratory